

Biochemical Facts behind the
Definition and Properties
of
METABOLITES

Edward D. Harris, Ph.D.
Professor
Biochemistry and Biophysics and
Faculty of Nutrition
Texas A&M University

Introduction

Investigations into the chemistry of living organisms have reached a stage where one can say with reasonable certainty that pathways of vitamins, minerals, amino acids and practically all components that comprise a living system are known. Thus, with identity to a known compound being a criterion, it is conceivable that there are scientific principles that will allow one to distinguish dietary ingredients that are in accord with the statutory meaning from those that fall outside that definition. However, there is a caveat, and that is that mere semblance in structure does not necessarily suffice to meet the criteria of a metabolite. The functionality of the molecule as it interacts within its natural environment must also be taken into account.

Scientific Considerations of Properties

Metabolites are the products of enzyme-catalyzed reactions that occur naturally within cells. To be classified as a metabolite a compound must meet certain criteria. Below is a summary of the major factors to consider in designating a substance a metabolite.

1. Metabolites are compounds found inside cells
2. Metabolites are recognized and acted upon by enzymes
3. The product of a metabolite must be able to enter into subsequent reactions
4. Metabolites have a finite half-life; they do not accumulate in cells
5. Many metabolites are regulators that control the pace of metabolism
6. Metabolites must serve some useful biological functions in the cell

Rationale for Metabolic Pathways and Metabolites

The concept of a "pathway" was put forward to give meaning to a seemingly endless series of chemical reactions that occur within living cells. The concept served to isolate a specific series of reactions that described logical changes to a parent compound. The intermediates that were formed in the pathway were referred to as "metabolites". Metabolites were thus compounds that intervened between the start and end of a pathway. Suffice to say, within the limits of certainty, pathways begin with defined compounds either derived directly from the blood stream or from an adjoining pathway¹.

A metabolite retains most if not all of the properties of its parent compound until it carbon structure blends into larger structures or is reduced to smaller structures. Examples of the former include amino acids that appear in proteins, glucose molecules that appear in glycogen,

fatty acids that appear in membrane lipids, or nucleotides that appear in DNA. Smaller metabolites are created when a parent compound is subject to systematic dismemberment as, for example, during oxidation reactions where the carbon appears in smaller size molecules and ultimately as carbon dioxide. These are examples where a metabolite is either a building block of a larger structure or a degradative product destined for excretion. Somewhat related are metabolites that enter different cellular compartments during the course of metabolism. Noting, for example, that a mitochondrial compound is derived from the cytosol allows one to designate that metabolite as an end product of a cytosolic pathway and the beginning compound of a mitochondrial pathway. The compound is still subject to change, but not within the compartment of its origin.

¹ Technically speaking, a compound outside the cell is not considered a metabolite. One definition would hold that metabolites arise by enzyme-catalyzed chemical changes within a cell. This is not a hard-fast rule. Glucose, for example, when in the blood is considered a metabolic product excreted from the cell but when inside the cell, glucose is a metabolite because of its vulnerability to chemical change.

Functions of Metabolic Pathways

As compounds course their way through pathways, the metabolic steps result in changes in chemical and biochemical properties. Oxidation reactions add oxygen or remove electrons, reducing agents add hydrogen, and other reactions transfer groups such as phosphate, amine, carboxyl, etc. to the parent structure. In essence the molecule is being transformed structurally. Catabolic reactions tend to split the molecule into component parts, whereas anabolic reactions tend to use the molecule to assemble larger structures. It should be noted that even though the parent molecule may no longer be visible, the carbon atoms that made up its structure can still give rise to additional compounds and thereby be properly considered as metabolites of that parent structure. *What understanding we lack is when during the course of such changes, the chemical identity has been eroded to such an extent that the molecule ceases to be identified with a unique parent structure and blends into the general milieu of molecules of other compounds.* Based on the aforementioned scenario, one must say that a definition of a metabolite must deem "proximity" to the parent structure as an important consideration in the definition.

Examples of Metabolites that Fit the Definition

Based on the limitations cited above, one can now turn to specific examples of parent-compound-metabolite and seek a rationale for rendering a decision.

Arachidonic acid and prostaglandins

Is arachidonic acid a metabolite of a prostaglandin? The answer is yes. Chemically, both molecules have nearly identical empirical formulae, both have very similar functional groups, both have very similar physical properties, both shun water and are soluble in non-polar solvents. Biochemically, arachidonic acid and the prostaglandin family are linked by a defined series of enzyme-catalyzed reactions that follow a logical progression of chemical change. The

separation perhaps involves no more than 5 distinct modifications to the prostaglandin molecule. This latter fits the requirement for proximity mentioned above. The modifications brought on by enzymes in the pathway result in a molecule that has acquired properties that are outside the scope of function of the parent compound. If the prostaglandin molecule continues to undergo changes in chemical composition, a point will be reached when it will be impracticable to determine by visual inspection alone when the derived molecule ceases to be a part of the parent structure, but instead blends with molecules derived from other pathways.

Inosine-5'-monophosphate (5'-IMP), PRPP, and glutamine

In this example 5'-IMP is a metabolite that has two source compounds. This is typical of many biochemical pathways, i.e., to have a metabolite arise by condensing two or more intermediates. It is important to note that in this example the reverse reaction does not occur, i.e., 5'-IMP cannot revert back to PRPP and glutamine. Directionality, dictated by principles of free energy exchange must, therefore, be taken into account. In biochemistry the free energy yield or requirement determines which direction the reaction goes and determines if a reverse of the forward reaction is possible. These energy relationships, therefore define precursor-product relationships in quantitative terms, making it possible to predict carbon flow, which in essence allows one to rule out certain compounds as precursors.

Steroid hormones and cholesterol

Steroid hormones are derived from cholesterol. These reactions constitute changes in the basic cholesterol nucleus that are irreversible. They are also characterized by minimal changes to the superstructure of the cholesterol ring. The derived products resemble cholesterol and share many of its physical properties such as solubility. The products, however, have acquired biochemical functionality that is not endowed within the cholesterol molecule. Examples of the latter include binding to receptor molecules in the cytosol and eliciting genetic expression in the nucleus. Cholesterol is assembled *de novo* from acetyl-CoA, a product of glucose and fat catabolism. In that reaction the smaller acetyl-CoA molecules coalesce to form intermediates of greater carbon number, very similar to a brick becoming a component of a wall. One could argue that acetyl-CoA loses its identity as it blends into the larger structure, or for that matter, in the first condensation reaction. The lesson here is that smaller metabolites will perhaps be the more difficult to define with precision because they rapidly lose any semblance of structure of the parent structures. Similarly, small metabolites can arise from the break down of numerous molecules whose origins are obscured by sheer numbers.

Catecholamine and tyrosine

This is an example of a defined pathway. Catecholamines such as L-DOPA, dopamine, norepinephrine, can only arise through the amino acid tyrosine. There is no alternative route. Hence, it is possible under such circumstances to state unequivocally a precursor-product relationship. It is also important to note that catecholamines cannot give rise to the aromatic amino acids from which they were formed. The pathway, therefore, is irreversible. Based on the established biochemistry, it is possible to state that for a compound to be a precursor of a

catecholamine, it must go through a tyrosine intermediate if it wishes to be indistinguishable from the natural compound. To bypass tyrosine is to postulate a non-biochemical event.

Concepts to Consider in Defining a Metabolite

"Just because it walks on two legs does not mean its human"

In arriving at a series of concepts for the properties of metabolites derived from dietary ingredients, the following are considered for discussion.

Concept 1: To be considered a metabolite, an ingredient in a dietary supplement must be closely identified with a known biochemical compound. If resemblance is not exact, there should be a known biochemical pathway that connects the unknown with the native molecule². Moreover, there should be limited number of feasible metabolic steps between the two and some assurance based on the chemistry that much of the architecture of the native molecule is in the ingredient molecule.

²Native refers to a compound that occurs naturally within the cell or its extracellular environment

Rationale: Structural similarity is one criterion used to link a dietary ingredient with a native compound. Evaluating and making judgements is based on a defined biochemical pathway. Thus if compound X, the ingredient, bears some structural resemblance to A, the closest naturally occurring biocompound, the two would appear to be metabolites in a common biochemical pathway. But, if X bears no resemblance to A or any other biocompound, an argument can be made against X being a metabolite. The validity of that argument must be based on (1) lack of a defined pathway between A and X, and (2) X showing few of the structural features of A. The latter presents the most difficult challenge because the further X is from A the less likely it will resemble A. For this reason, X and A should be within a reasonable number of metabolic steps apart. If X undergoes hydrolysis or condensation to form A, the absolute origin of X cannot be determined with certainty and its relation to a parent compound becomes arbitrary. Hydrolysis destroys chemical structure and when the hydrolysis products are part of a general pool, tracing the origin of the compound becomes impossible.

Concept 2: Derived metabolites must not represent a threat to homeostatic mechanisms.

A metabolite is synthesized by the cell for the purpose of performing (1) a biological function as is, or (2) a biochemical function after a structural modification, or (3), no biological function per se other than to be excreted as a waste product. The principle recognizes that metabolites have a purpose that is designed to benefit, not harm cells.

Rationale: Metabolites tend to fall into two categories. Those that are generally present in abundance and those that are present in fleeting, and sometimes non-detectable amounts. An increase in the first category would have little perturbation on cell function. Metabolites present in fleeting amounts, however, do not accumulate, because their destiny is to be changed and their low concentration is indicative of the rapidity of that process. In essence, category two molecules are sacrificial. An important function of category two molecules is regulation. Suffice to say

that a metabolite that is a metabolic regulator (see concept 5) when present in very small concentrations has the capacity to exert profound effects by slight changes in concentrations. Within this scenario, one can see that harm can arise by perturbing the balance of minor metabolites within a pathway. Perturbing balance could mean:

1. Reversing carbon flow to favor degradation over synthesis or vice versa. On a larger scale this could mean advancing one process at the expense of another.
2. Stimulating production of inducible enzymes. Enzyme concentrations tend to keep the status quo. Metabolic flow can be interrupted by changes in the activity of these important pace setters.
3. Altering the activity of allosteric enzyme. Allosteric enzymes monitor pathways by instantaneous recognition of changes in some key metabolite.
4. Effecting a rate determining step(s). This has been alluded to earlier. A rate-determining step controls carbon flux. The pathway moves no faster than the concentration of the limiting component.

Concept 3: Metabolites must perform useful, if not indispensable functions to cell survival

Rationale: The inference of this rule is obvious. Regardless of concentration, a metabolite is present for a purpose that is linked closely with survival. That purpose may be to contribute to the infrastructure or energy requirement of the cell.

Concept 4: Metabolites have biochemical compatibility with the normal processes of synthesis and replacement.

Rationale: A characteristic of a living system is the continual replacement of worn out or spent molecules. This is especially true of macromolecules whose half-life can be measured in seconds, minutes and hours. Metabolites do not accumulate because such would upset a delicate balance of metabolic turnover. In essence, metabolites are synthesized to be used, to have a finite time of function, and to be compatible with systems that replace them.

Concept 5: Metabolites can serve as regulators of carbon flow in competing and interacting pathways.

Rationale: This concept does not apply to all metabolites. Regulation of metabolic events occurs at the level of pathway intermediates. Certain metabolites exert profound effects on biochemical processes through their ability to feedback and inhibit (or stimulate) the action of enzymes in the pathway. Biochemists are alert to a family of enzymes referred to as "allosteric". The principle of allostery when applied is best described as a self-governing system that controls the direction and rate of a metabolic pathway by influencing the activity of rate-determining enzymes in the pathway. On rare occasions a metabolite can also influence synthesis of enzymes

de novo by interacting at the genetic level of cell function. These concepts point to important functions of metabolites in maintaining the pace of metabolism.

Summary

Metabolites are the byproducts of metabolism. Their formation critically depends on enzymes that act on a parent structure. As such, they represent defined chemical intermediates in a pathway that is designed to modify the parent compound. Because they are intermediates, metabolites tend to be present in small amounts. Nonetheless, the metabolites that have been inferred to exist in biochemical pathways have had their existence confirmed by rigorous chemical analysis of the isolated compounds. Metabolites, therefore, are not hypothetical constructs used to explain how compound A became compound B. A characteristic of a metabolite is rapid chemical change. This is especially true if the metabolite is an intermediate in a pathway where stability considerations must enter into the discussion. A metabolite owes its instability to the enzyme that will use the metabolite as a substrate for a subsequent step in the pathway. It is not unusual for metabolites to be "channeled" into pathway enzymes due to close packing as in a membrane bound system. Metabolites have a finite existence in a cell and generally are not allowed to accumulate. Metabolic turnover is a principle of life and the synthesis and degradation of metabolites is one of the ways turnover is accomplished. Some metabolites have the capacity to act directly on enzymes or on the transcription machinery of a cell. In both cases the metabolite can influence its own metabolic fate and the fate of molecules in a pathway of which it is a member.

Conclusions and Considerations

To fit the definition of a metabolite, a dietary ingredient must bear a semblance to a "living" molecule both in structure and function. The candidate molecule(s) must interact favorably with the host's metabolic machinery. Enhancing the level of the candidate molecule must not present a toxic challenge to the host or otherwise cause an untold situation that would differ from the elevation of the host's molecule. The candidate molecule must have a known metabolic fate that does not violate the principles of metabolic turnover. The candidate molecule must behave in accordance with the principles of metabolic turnover. It must show timely degradation and excretion. It must not leave a lasting imprint on the metabolic systems of the host or cause the host system to adapt to a new position of homeostasis or need. In essence, it must not be addictive. Molecules fitting these criteria, or supplement ingredients that give rise to said molecules, would be considered capable of meeting the host need for optimal health, growth and development.

Based on the concepts enunciated above, any compound that serves as a dietary ingredient must demonstrate that imparts a vital function to cells. Failure to do so risks putting cells into a downward spiral toward apoptosis, or programmed cell death. Although product safety and efficacy is not an issue to be addressed, it behooves anyone who would recommend a

particular supplement to provide evidence that the supplement contains no ingredients that will upset a delicate balance of ongoing metabolism or delay the natural turnover of components, a process that is characteristic for sustaining life.